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ENCLOSURE TO AMENDMENT AND REMARKS

J Nucl Med. 1976 Jan;17(1):40-6.
In vivo behavior of 99mTc-fibrinogen and its potential as a thrombus-imaging agent.
Harwig SS, Harwig JF, Coleman RE, Welch MJ.

We have investigated the in vivo behavior of 99mTc-fibrinogen, prepared by a mild and efficient electrolytic method employing tin electrodes. The clearance mechanisms of this agent were studied, and its efficacy for imaging deep-vein thrombi in dogs with an Anger camera was determined. The 99mTc-fibrinogen preparations, which are stable in vitro, undergo partial rapid exchange of the technetium with other plasma proteins and with anions of the blood buffer system in vivo, resulting in an early drop in the percent of radioactivity associated with clottable protein. However, very little or no oxidation to pertechnetate occurs. The nonclottable material is much more rapidly cleared from the blood than the remaining 99mTc-fibrinogen, and the proportion of clottable protein activity increases with time. The fraction of 99mTc-fibrinogen that remains intact in vivo is biologically active and will incorporate into thrombi. Higher thrombus-to-blood activity ratios are obtained with 99mTc-fibrinogen than with radioidinated fibrinogen when both agents are injected into dogs 4 hr after induction of femoral vein thrombosis. Clearly delineated images of the thrombi are obtained, beginning about 2.5 hr after injection. Thus, 99mTc-fibrinogen may be of clinical use as a thrombus-imaging agent in patients under-going active thrombosis; especially in regions of high blood pool.

Eur J Nucl Med. 1978;3(4):233-8.
The interpretation of phlebograms using fibrinogen labeled with 99 mTc.
Jonckheer MH, Abramovici J, Jeghers O, Dereume JP, Goldstein M.

A new method for the detection of deep-vein thrombosis is presented, consisting of a single antecubital injection of fibrinogen labeled with 99mTc. This atraumatic procedure allows one to

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visualize the large veins of the lower limbs, the venae iliacae and the distal part of the vena cava inferior. This paper discusses how to interpret these phlebograms.

Bioconjug Chem. 1997 Mar-Apr;8(2):155-60.
Biological evaluation of thrombus imaging agents utilizing water soluble phosphines and tricine as coligands when used to label a hydrazinonicotinamide-modified cyclic glycoprotein IIb/IIIa receptor antagonist with 99mTc.
Barrett JA, Crocker AC, Damphousse DJ, Heminway SJ, Liu S, Edwards DS, Lazewatsky JL, Kagan M, Mazaika TJ, Carroll TR.

A hydrazinonicotinamide-functionalized cyclic glycoprotein IIb/IIIa (GPIIb/IIIa) receptor antagonist [cyclo(D-Val-NMeArg-Gly-Asp-Mamb(5-(6-(6-hydrazinonicotin amido)hexanamide))) (HYNICtide)] was labeled with 99mTc using tricine and a water soluble phosphine [trisodium triphenylphosphine-3,3',3"-trisulfonate (TPPTS); disodium triphenylphosphine-3,3'-disulfonate (TPPDS); or sodium triphenylphosphine-3-monosulfonate (TPPMS)] as coligands. Three complexes, [99mTc(HYNICtide)(L)(tricine)] (1, L = TPPTS; 2, L = TPPDS; 3, L = TPPMS), were evaluated in the canine arteriovenous shunt (AV shunt) model and canine deep vein thrombosis imaging (DVT) model. All three agents were adequately incorporated into the arterial and venous portions of the growing thrombus (7.8-9.9 and 0.2-3.7% ID/g, respectively) in the canine AV shunt model. In the canine DVT model all three complexes had thrombus uptake that far exceeded the negative control, [99mTc]albumin. The findings indicate similar incorporation into a venous thrombus (% ID/g = 2.86 +/- 0.4, 3.4 +/- 0.9, and 3.38 +/- 1.1 for complexes 1, 2, and 3, respectively) and similar blood clearance with a t1/2 of approximately 90 min. Gamma camera scintigraphy allowed visualization of deep vein thrombosis in as little as 15 min with the thrombus/muscle ratios being 3.8 +/- 0.8, 2.8 +/- 0.4, and 3.0 +/- 0.8 for complexes 1, 2, and 3, respectively. The visualization of the thrombus improved over time, and the thrombus/muscle ratios were 9.7 +/- 1.9, 13.8 +/- 3.6, and 9.4 +/- 2 for complexes 1, 2, and 3, respectively, at 120 min postinjection. The administration of complexes 1-3 did not alter platelet function, hemodynamics, or the coagulation cascade. Furthermore, complexes 1-3 did not significantly differ in their uptake into the growing thrombus, blood clearance, and target to background ratios. Therefore, all three complexes have the capability to detect rapidly growing venous and arterial thrombi.

Coron Artery Dis. 1998;9(2-3):131-41.
Novel technetium-99m-labeled platelet GPIIb/IIIa receptor antagonists as potential imaging agents for venous and arterial thrombosis.
Mousa SA, Bozarth JM, Edwards S, Carroll T, Barrett J.

OBJECTIVES: Either venous or arterial thrombosis is a potentially life-threatening event and existing diagnostic modalities are inadequate to diagnose and to determine the morphology of the evolving thrombus. Thus development of a noninvasive imaging agent that can detect clot location remains a critical and unmet need in nuclear diagnostic medicine. The present study was undertaken to determine the potential of platelet GPIIb/IIIa receptors compared with direct thrombin inhibitors, in the detection of venous and arterial clots. METHODS: Initially, the

validity of exploiting the degree and extent of specific uptake and retention of a potent GPIIb/IIIa receptor antagonist in venous and in arterial thrombus was confirmed in vitro in artificially created arterial- or venous-type clots, using the radiolabeled antagonist, 3H-DMP728. This was followed by comparing the in-vivo clot/blood distribution of various technetium-99m (99mTc)-labeled, DMP728-derived, GPIIb/IIIa receptor antagonists and of thrombin inhibitors, over time, in mixed arterial/venous or venous clots in arteriovenous shunt and in venous clot models in dogs. In addition, we performed noninvasive single-photon emission tomographic imaging of the venous clot in a deep vein thrombosis model in dogs. RESULTS: Our data confirmed that potency for the platelet GPIIb/IIIa receptors was maintained after radiolabeling of the parent active GPIIb/IIIa receptor antagonists. DMP728 demonstrated a relatively greater affinity for activated than for unactivated human platelets, which might be essential for attaining an optimal thrombus/blood (target/background) distribution ratio and the optimal detection of small clots (i.e. greater sensitivity). CONCLUSIONS: These data suggest a potential utility of 99mTc-GPIIb/IIIa receptor antagonists, but not of direct thrombin inhibitors, in the diagnosis of venous clots in deep vein thrombosis, pulmonary embolism and arterial thromboembolic disorders including stroke and coronary and peripheral artery thrombotic disorders.